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THE ROLE OF SODIUM CHLORIDE IN SEQUENTIAL INDUCTION OF THE PRESUMPTIVE EPIDERMIS OF RANA PIPIENS GASTRULAE[1]

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Previous investigations have shown that ions will induce various cell types from the presumptive epidermis of the *Rana pipiens* gastrula (Barth and Barth, 1959, 1962, 1963, 1964, 1966). When it was found that sucrose also would induce (Barth, 1965), the question arose as to whether sucrose acted directly as an inductor or indirectly by facilitating the penetration of ions. A study of the effects of sucrose in relation to various concentrations of sodium chloride was undertaken.

Methods

The solutions and procedures used for operation, treatment, and culture of small aggregates of cells from the *Rana pipiens* embryo have been described in detail (Barth and Barth, 1959, 1962, 1963, 1964, 1966). Essentially the procedure consists of the following steps: (1) Presumptive epidermis regions, for example, are dissected out in standard solution and treated briefly with Versene (EDTA) to loosen the pigment coat layer from underlying presumptive epidermis cells; (2) aggregates consisting of approximately 100 cells each are teased out from the presumptive epidermis and allowed to heal for 10–15 minutes before transfer to treatment or culture solutions; (3) cultures of such aggregates in small glass stender dishes prepared and maintained under sterile conditions are able to be observed daily in the living condition.

Results

Table I records the data obtained with sucrose substituted for the sodium chloride in the standard solution used for culture of the presumptive epidermis. Low concentrations of sucrose (exps. 1 and 2) induce radial nerve and slate gray epithelium, while higher concentrations (exps. 7, 8 and 9) induce pigment cells and nerve. The higher concentrations applied for different periods of time induce first nerve, then slate gray epithelium and finally pigment cells (exp. 11). Thus,

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TABLE I

Sequential induction by solutions in which NaCl is replaced by sucrose

NaCl is omitted from the medium and varying amounts of sucrose added in its place.
In this and succeeding tables the headings are to be interpreted as follows:
Stage no.: Shumway (1940); conc.: concentration of substances in milligrams per milliliter of solution; hrs.: time in hours during which aggregates are exposed to the substances indicated; types of cellular differentiation: as in Barth and Barth (1962, 1963, 1964); UPS = unipolar spongioblasts.

Exp. no.	Stage no.	Treatment		No. of aggregates	Types of cellular differentiation
		Conc.	Hrs.		
1	11	29.0	6.0	25	Radial nerve
	11	29.0	8.5	42	Radial nerve
2	11	43.0	3.3	75	Radial nerve, epithelium
	11	43.0	6.3	75	Nerve, slate gray epithelium, epithelium
3	11−	43.0	4.0	40	Nerve
	11−	43.0	5.5	35	Nerve, pigment cells
4	11+	58.0	2.5	35	Nerve, rare pigment cells
5	11	58.0	4.5	75	Nerve, pigment cells
6	11	58.0	5.0	75	Nerve, pigment cells
7	11−	58.0	5.0	70	Nerve, pigment cells
8	11−	58.0	6.0	75	Nerve, pigment cells
9	11	58.0	9.5	50	Pigment cells, slate gray epithelium, nerve
10	11	58.0	5.0	75	Nerve, slate gray epithelium, UPS
	11	58.0	10.5	75	Pigment cells, nerve
11	11	58.0	3.0	25	Nerve, UPS
	11	58.0	6.0	50	Nerve, slate gray epithelium, UPS
	11	58.0	8.5	75	Pigment cells, nerve
12	11	65.0	4.5	75	Nerve, pigment cells
13	11−	65.0	6.0	75	Nerve, pigment cells
14	11−	87.0	4.0	60	Nerve
15	11−	116.0	3.0	28	Nerve
	11−	116.0	4.0	32	Dead

sucrose appears to induce sequentially a variety of cell types as do various ions (Barth, 1965).

Since the above experiments were done with a medium containing the normal concentrations of K^+, Ca^{++}, Mg^{++}, HCO_3^- and phosphate ions, any of these ions

might have acted as the inductor. Therefore sucrose was applied to the cells in the complete absence of ions.

Table II records the results of experiments in which various concentrations of sucrose dissolved in glass-distilled water were applied to presumptive epidermis for varying lengths of time. Experiment 1 shows that a low concentration of sucrose induces nerve and slate gray epithelium. Higher concentrations (exps. 3, 4 and 7) induce pigment cells and nerve. Experiments 2 and 6 show sequential induction of radial nerve, spreading nerve and unipolar spongioblasts, while in experiment 3 with longer times of exposure pigment cells are induced. Thus, sucrose in the complete absence of ions will induce the various cell types which are also induced by ions.

TABLE II

Sequential induction by sucrose in glass-distilled water

Exp. no.	Stage no.	Treatment		No. of aggregates	Types of cellular differentiation
		Conc.	Hrs.		
1	11 –	43.0	5.0	28	Nerve, slate gray epithelium
2	11	58.0	0.5	20	Ciliated epithelium
	11	58.0	0.75	20	Radial nerve, ciliated epithelium
	11	58.0	1.0	20	Spreading nerve
	11	58.0	1.5	20	Spreading nerve, UPS
3	11	58.0	1.0	25	Nerve, pigment cells
	11	58.0	2.0	25	Pigment cells, nerve
	11	58.0	2.5	25	Pigment cells, nerve, dead cells
4	11+	58.0	2.5	28	Pigment cells, nerve
5	11 –	58.0	0.75	40	Spreading nerve, UPS
6	11 –	58.0	0.1	35	Ciliated masses
	11 –	58.0	0.5	40	Nerve, ciliated epithelium
	11 –	58.0	1.25	35	Nerve, ciliated epithelium
	11 –	58.0	1.75	40	Nerve, UPS
7	12 –	58.0	0.4	40	Spreading nerve, radial nerve
	12 –	58.0	1.5	36	Nerve, slate gray epithelium, pigment cells
8	11	68.0	1.0	40	Spreading nerve, slate gray epithelium, pigment cells
	11	68.0	1.7	35	Spreading nerve, pigment cells
9	11	90.0	1.0		Spreading nerve, slate gray epithelium
	11	90.0	1.7		Spreading nerve
10	11	137.0	1.0		Spreading nerve, radial nerve
	11	137.0	1.5		Spreading nerve, UPS
	11	137.0	2.0		Spreading nerve, UPS
	11	137.0	3.0		Dead cells

TABLE III

Induction by sugars other than sucrose, dissolved in glass-distilled water
gl = glucose; lac = lactose; xyl = xylose.

Exp. no.	Stage no.	Treatment		No. of aggregates	Types of cellular differentiation
		Conc.	Hrs.		
1	11	lac 58	4.5	35	Pigment cells
	11	lac 58	5.1	40	Pigment cells
2	11 –	lac 58	4.0	28	Pigment cells
	11 –	lac 58	4.5	32	Pigment cells
3	11 –	gl 31	2.0	75	Spreading nerve, UPS
	11 –	gl 31	3.0	75	Spreading nerve, UPS, slate gray epithelium
4	11 –	gl 31	4.0	32	Pigment cells
	11 –	gl 31	4.5	20	Pigment cells
5	11	gl 31	4.5	40	Pigment cells
	11	gl 31	5.1	25	Pigment cells
6	11	xyl 20	0.5		Nerve, ciliated masses
	11	xyl 20	1.0		Spreading nerve
	11	xyl 20	2.0		Pigment cells, slate gray epithelium

The next question asked was: Is sucrose peculiar in some respect as regards induction, or will other sugars induce? Table III records the results of experiments with other sugars when these compounds are dissolved in glass-distilled water. Experiments 1 and 2 show that lactose will induce pigment cells, while experiments 3, 4 and 5 demonstrate the sequential action of glucose when applied for different intervals of time. Experiment 6 shows sequential induction by xylose with respect to time. Thus sucrose is not unique in its ability to induce but shares this quality with lactose, glucose and xylose.

Speculations on the nature of the action of sugars as inductors led to a number of possible actions. First of all the sugars could be acting directly as inductors by some unknown mechanism or they could act indirectly by altering the cells in some manner so that subsequently the ions of the standard solution would induce. The possibility that the ions of the standard solution might induce is indicated by the fact that in some species of Amphibia, namely *Ambystoma maculatum* and *Ambystoma opacum,* presumptive epidermis will differentiate into brain without the presence of extraneous inductors (Barth, 1941; Holtfreter, 1944). Therefore the standard salt solution must have been the inductor. We also had some evidence that some substances would induce if applied for a short time, while long periods of treatment resulted in ciliated epidermis. Such a finding would be consistent with the hypothesis that induction occurred in the standard salt solution and not in the substance tested. This follows from the known fact that ability of cells to be induced by an inductor disappears with time.

Experiments therefore were set up to test the hypothesis that sugars did not

actually induce but that the induction occurred when the cells were transferred to the standard salt solution.

Two types of experiments were designed to test the idea. In one, the cells were to be kept in sucrose until the period of competence was concluded and then returned to the standard solution. Under these conditions no induction was to be expected. The other type of experiment consisted in reducing the ionic strength of the standard solution in an attempt to reduce its inductive capacity. The first type of experiment proved to be too difficult to carry out, as the cells would not survive sucrose treatment in absence of ions when exposed during the entire period of competence. The second type of experiment gave definite results.

Table IV records the results of experiments in which the ionic strength of the standard salt solution was reduced by varying the concentration of sodium chloride. The standard solution contains 5.15 mg. NaCl/ml. Experiment 1 shows that a concentration of 1.28 mg./ml. is too low for continuous treatment of the cells, although ciliated masses will develop after 7.5 hours tratment. Experiments 2 and 3 show that 2.0 mg./ml. is the minimum concentration of sodium chloride which may be used for continuous culture of the cells. Experiments 4–8 record the results with 2.55, 2.57 and 3.0 mg./ml. of sodium chloride.

The first results of the effect of a reduction in ionic strength upon induction are shown in Table V, experiments 1, 2 and 3. There was no induction by sucrose

TABLE IV

The effect of various concentrations of sodium chloride

Normal concentration is 5.15 mg./ml. Other ions are present in normal concentration.

Exp. no.	Stage no.	Treatment		No. of aggregates	Types of cellular differentiation
		Conc.	Hrs.		
1	11	1.28	1.5	25	Radial nerve, spreading nerve, little ciliated epithelium
	11	1.28	7.5	25	Ciliated masses, many unattached single cells
	11	1.28	cont.	25	Dead cells
2	11	2.00	7.5	30	Masses with voluminous mucus, some cilia
	11	2.00	cont.	20	Ciliated masses with voluminous mucus
3	11–	2.00	6.0	25	Ciliated masses with some mucus
	11–	2.00	cont.	20	Ciliated masses with voluminous mucus
4	11	2.55	3.0	25	Ciliated epithelium
	11	2.55	6.0	25	Ciliated epithelium
	11	2.55	8.5	25	Ciliated epithelium
5	11	2.57	6.0	25	Ciliated masses with some mucus
	11	2.57	cont.	25	Ciliated masses, ciliated epithelium
6	11	2.57	cont.	25	Ciliated masses
7	11	2.57	cont.	25	Ciliated masses with a little mucus, ciliated epithelium
8	11–	3.00	cont.	25	Ciliated epithelium

TABLE V

Lack of induction by sucrose when followed by culture in low concentrations of sodium chloride

Sucrose dissolved in glass-distilled water. Standard salt solution contains 5.15 mg. NaCl/ml. Other ions are present in same concentrations as in our standard solution.

Exp. no.	Stage no.	Sucrose treatment		No. of aggregates	NaCl conc. in culture	Types of cellular differentiation
		Conc.	Hrs.			
1	11–	58.0	1.25	35	5.15	Spreading nerve, UPS
	11–	58.0	1.25	40	2.00	Ciliated masses, mucus
2	11–	58.0	2.0	35	5.15	Spreading nerve, UPS
	11–	58.0	2.0	40	2.0	Ciliated masses
3	11–	58.0	1.0	38	2.0	Ciliated masses
	11–	58.0	1.5	40	2.0	Ciliated masses
	11–	58.0	2.0	35	2.0	Ciliated masses
	11–	58.0	2.3	35	2.0	Ciliated masses
4	11	58.0	2.0	25	2.57	Ciliated masses
	11	58.0	2.5	25	2.57	Ciliated masses, mucus
	11	58.0	3.0	25	2.57	Ciliated masses, mucus
5	11	58.0	2.0	40	2.57	Ciliated masses
6	11	58.0	2.0	40	2.57	Ciliated masses, little mucus, ciliated epithelium
7	11–	58.0	2.0	40	2.57	Ciliated masses
8	11	58.0	2.0	35	2.57	Ciliated masses
9	11	58.0	2.3	25	2.57	Ciliated masses, mucus, ciliated epithelium
10	11	58.0	2.2	40	2.57	Ciliated masses, ciliated epithelium
11	11	58.0	2.8	25	2.57	Ciliated masses, mucus, ciliated epithelium
12	11	58.0	2.3	25	2.57	Ciliated masses, mucus, ciliated epithelium
13	11	58.0	1.8	40	3.00	Spreading nerve, radial nerve
14	11–	59.0	2.3	40	2.57	Ciliated epithelium
	11–	59.0	2.3	40	3.50	Spreading nerve
	11–	59.0	2.8	40	2.57	Ciliated epithelium, rare nerve
	11–	59.0	2.8	40	3.50	Spreading nerve, UPS
15	11–	58.0	4.0	25	3.75	Spreading nerve
16	11	58.0	2.7	35	4.00	Spreading nerve, pigment cells

when the cells were returned to 2.0 mg./ml., but good induction when they were returned to 5.15 mg./ml. Experiments 4 through 12 resulted in no induction with sucrose when the cells were returned to sodium chloride at a concentration of 2.57

mg./ml. If, after sucrose treatment, the cells were returned to a solution containing 3.0 mg./ml., induction of radial nerve and spreading nerve took place. Experiment 14 shows that induction occurs in a solution containing 3.50 mg. of sodium chloride per milliliter but not at a concentration of 2.57 mg./ml. At concentrations of 3.75 and 4.0 mg./ml. induction also occurs (exps. 15 and 16).

It is clear that induction with sucrose is dependent upon the concentration of sodium chloride in the solution into which the cells are subsequently transferred. Table VI records the data from experiments in which after treatment with sucrose the cells were transferred to different concentrations of sodium chloride. The extent of induction is proportional to the concentration of sodium chloride.

Either the cells are not induced by sucrose or the cells are induced by sucrose but cannot differentiate in low concentrations of sodium chloride. Table VII demonstrates that induced cells do differentiate in low concentrations of sodium chloride. In these experiments the cells are first treated with sucrose, then transferred to high concentrations of sodium chloride for varying periods of time, and lastly transferred to low sodium chloride for culture. Experiment 1 shows that while a two-hour post-treatment with high sodium chloride (4.25 mg./ml.) results mostly in ciliated cells with a little radial nerve present, a five-hour post-treatment

TABLE VI

After treatment with sucrose, induction is proportional to the concentration of NaCl in the culture medium

Sucrose dissolved in glass-distilled water. Concentration of NaCl is varied, but other ions are as in our standard solution.

Exp. no.	Stage no.	Sucrose treatment		No. of aggregates	NaCl conc. in culture	Types of cellular differentiation
		Conc.	Hrs.			
1	11–	58.0	2.0	35	2.57	Ciliated masses
	11–	58.0	2.0	40	3.00	Ciliated masses, radial nerve, spreading nerve
	11–	58.0	2.0	35	3.50	Spreading nerve, radial nerve
	11–	58.0	2.0	40	4.00	Spreading nerve, UPS
2	11–	58.0	2.1	35	2.57	Ciliated masses, mucus
	11–	58.0	2.1	40	3.00	Ciliated masses, spreading nerve
	11–	58.0	2.1	40	4.00	Spreading nerve, UPS, slate gray epithelium, pigment cells
3	11–	58.0	2.5	30	2.00	All ciliated masses
	11–	58.0	2.5	30	2.57	Ciliated masses, rare nerve
	11–	58.0	2.5	30	3.00	Ciliated masses, ciliated epithelium, nerve
	11–	58.0	2.5	30	3.75	Nerve, no ciliated cells
	11–	58.0	2.5	30	4.50	Spreading nerve, UPS
4	11	58.0	2.1	25	2.25	Ciliated masses, mucus
	11	58.0	2.1	25	2.57	Ciliated masses
	11	58.0	2.1	25	3.00	Nerve, cilia rare
	11	58.0	2.1	25	3.50	Spreading nerve, UPS
	11	58.0	2.1	25	3.50	UPS, spreading nerve

TABLE VII

After pre-treatment with sucrose, induction is proportional to the time of exposure to high concentrations of NaCl

Aggregates are pre-treated with sucrose in glass-distilled water, then transferred to a "high" concentration of NaCl for varying lengths of time (post-treatment), and finally transferred to a culture medium of "low" NaCl content. "High" concentration is from 4.25 to 5.15 mg. of NaCl per ml. of solution containing the other ions in normal concentrations.

Exp. no.	Stage no.	Sucrose pre-treatment		No. of aggregates	NaCl post-treatment		NaCl conc. in culture	Types of cellular differentiation
		Conc.	Hrs.		Conc.	Hrs.		
1	11 –	58.0	2.0	35	4.25	2.0	2.57	Ciliated masses, ciliated epithelium, radial nerve
	11 –	58.0	2.0	40	4.25	5.0	2.57	Spreading nerve, no cilia
	11 –	58.0	2.0	40	4.25	7.0	2.57	Spreading nerve, no cilia
	11 –	58.0	2.0	35	4.25	19.0	2.57	Spreading nerve, no cilia
2	11	58.0	2.3	25	—	—	2.25	Ciliated masses
	11	58.0	2.3	25	4.25	1.0	2.25	Ciliated masses
	11	58.0	2.3	25	4.25	2.0	2.25	Nerve, ciliated masses
	11	58.0	2.3	25	4.25	5.0	2.25	Nerve, no cilia
	11	58.0	2.3	25	4.25	19.0	2.25	Nerve, no cilia
3	11 –	54.0	2.0	35	5.15	0.2	2.57	Ciliated masses, rare nerve
	11 –	54.0	2.0	40	5.15	2.0	2.57	Spreading nerve, no cilia
	11 –	54.0	2.0	40	5.15	4.5	2.57	Extensive spreading nerve, no cilia
	11 –	54.0	2.0	40	5.15	19.5	2.57	Extensive spreading nerve, no cilia
4	11 –	58.0	2.0	40	5.15	19.0	2.57	Nerve, no cilia
5	11 –	58.0	2.0	50	5.15	19.0	2.57	Nerve, no cilia

induces spreading nerve. In experiment 3 where post-treatment consisted of 5.15 mg. of sodium chloride per milliliter of solution, induction occurred in two hours. The extent of induction is proportional to the time of post-treatment with high concentrations of sodium chloride.

It is clear that sucrose will not induce unless followed by a treatment with a solution containing from 3.0 to 5.15 mg. of sodium chloride. Do sucrose and sodium chloride have similar effects so that they are synergetic or does sucrose merely prepare the cells for induction by sodium chloride? Table VIII records the results of experiments designed to answer this question. Experiment 1 shows that 29.0 mg. of sucrose will induce nerve while 2.57 mg. of sodium chloride has no inductive properties. The two compounds in combination have no inductive properties. Sodium chloride, therefore, applied simultaneously with sucrose antagonizes the inductive action of sucrose and the cells differentiate into ciliated masses instead of nerve.

Experiment 2 shows that 29.0 mg. of sucrose applied for 8 hours will induce as far as pigment cells, but when combined with sodium chloride the sucrose has no inductive ability. Experiments 3, 4, 5 and 6 confirm and extend the results of exps. 1 and 2.

Therefore sucrose cannot induce by itself unless followed by high sodium chloride, nor can sucrose in combination with sodium chloride induce regardless of subsequent treatment. It may be concluded, therefore, that the action of sucrose in the absence of sodium chloride is to alter the cell surfaces so as to permit sodium chloride and other ions to penetrate.

Actually the alteration of the cell surfaces is probably due to *lack* of sodium chloride in the solution and not to the presence of sucrose. Sucrose probably merely maintains the osmotic pressure necessary for survival of cells while lack of sodium chloride produces the alteration in the cell surfaces. The fact that

TABLE VIII

The antagonism of sucrose and NaCl when applied together

In these experiments the concentrations of all ions except Na^+ and Cl^- are kept constant. Various concentrations of NaCl (Na) and/or sucrose (S) are added to a standard solution lacking NaCl. Culture of the aggregates after treatment is also in the presence of the normal concentrations of all ions except Na^+ and Cl^-.

Exp. no.	Stage no.	Treatment concentrations	Hrs.	No. of aggregates	Culture	Types of cellular differentiation
1	11	29 S	6.0	25	5.15 Na	Nerve
	11	29 S	6.0	25	2.57 Na	Nerve, ciliated masses
	11	2.57 Na	6.0	25	2.57 Na	Ciliated masses, some mucus
	11	2.57 Na	6.0	25	5.15 Na	Ciliated masses, ciliated epithelium
	11	29 S + 2.57 Na	6.0	25	2.57 Na	Ciliated masses, some mucus
	11	29 S + 2.57 Na	6.0	25	5.15 Na	Ciliated epithelium
2	11 –	29 S	3.0	20	5.15 Na	Nerve
	11 –	29 S	3.0	20	2.57 Na	Nerve
	11 –	29 S + 2.57 Na	3.0	20	5.15 Na	Ciliated epithelium
	11 –	29 S + 2.57 Na	3.0	20	2.57 Na	Ciliated masses
	11 –	29 S	8.0	20	5.15 Na	Nerve
	11 –	29 S	8.0	20	2.57 Na	Pigment cells, nerve
	11 –	29 S + 2.57 Na	8.0	20	5.15 Na	Ciliated epithelium, radial nerve
	11 –	29 S + 2.57 Na	8.0	20	2.57 Na	Ciliated masses
3	11	29 S + 2.57 Na	5.3	25	5.15 Na	Ciliated masses
	11	29 S + 2.57 Na	5.3	25	29 S + 2.57 Na	Ciliated masses
4	11	29 S	6.0	25	5.15 Na	Radial nerve, spreading nerve, UPS
	11	29 S	8.0	40	5.15 Na	Radial nerve, spreading nerve, UPS
	11	29 S	3.0	25	5.15 Na	Radial nerve
	11	2.55 Na	3.0	25	5.15 Na	Ciliated epithelium
	11	2.55 Na	6.0	25	5.15 Na	Ciliated epithelium
	11	2.55 Na	8.0	25	5.15 Na	Ciliated epithelium
5	11	29 S + 2.55 Na	9.5	50	5.15 Na	Ciliated epithelium
	11	5.15 Na	9.5	50	5.15 Na	Ciliated epithelium
	11	58 S	9.5	50	5.15 Na	Pigment cells, nerve, slate gray epithelium
6	11	34 S + 5.15 Na	2.0	35	5.15 Na	Ciliated masses
	11	34 S + 5.15 Na	5.5	40	5.15 Na	Ciliated masses
	11	34 S + 5.15 Na	18.0	75	5.15 Na	Ciliated masses

TABLE IX

Correlation between pre-treatment with solutions lacking sodium chloride and induction by standard salt solution

Sodium chloride is omitted from the standard salt solution and other substances are added with or without sodium chloride. After a period of treatment the aggregates are transferred to standard solution containing 5.15 mg. NaCl/ml. E.G. = ethylene glycol; gl = glycine; suc = sucrose.

Exp. no.	Stage	Treatment concentrations	hrs.	No. of aggregates	Types of cellular differentiation
1	11	13 gl	0.5	30	Spreading nerve, ciliated masses
	11	13 gl	1.0	30	Spreading nerve
	11	13 gl	1.7	30	Dead cells, spreading nerve
	11	13 gl	3.0	10	Dead cells
2	11	31 E.G.+2.0 NaCl	1.0	25	Radial nerve, spreading nerve, epithelium
	11	31 E.G.+2.0 NaCl	2.0	25	Epithelium, ciliated masses, mucus
	11	31 E.G.+2.0 NaCl	4.0	25	Ciliated masses, voluminous mucus
	11	31 E.G.+5.15 NaCl	0.3	25	Ciliated masses, mucus, epithelium, nerve
	11	31 E.G.+5.15 NaCl	5.0	25	Ciliated masses, mucus
	11	31 E.G.	1.0	25	Dead cells
	11	31 E.G.	2.0	25	Dead cells
	11	6 E.G.+5.15 NaCl	1.5	30	Ciliated masses, mucus
	11	6 E.G.+5.15 NaCl	6.0	35	Ciliated masses, mucus
	11	6 E.G.+5.15 NaCl	16.0	25	Ciliated masses, mucus
3	11	0.0 NaCl	0.25	35	Ciliated masses, epithelium, rare nerve
	11	0.0 NaCl	0.5	20	Nerve, epithelium, rare UPS
	11	0.0 NaCl	0.75	12	Spreading nerve
	11	0.0 NaCl	3.0	50	Cytolyzed
	11	58 suc	0.75	40	Spreading nerve, UPS
	11	0.65 NaCl	0.75	25	Spreading nerve, epithelium
	11	0.65 NaCl	1.0	25	Nerve, epithelium

sodium chloride added to sucrose results in no induction suggests strongly that the induction is by means of a lack of sodium chloride.

Additional evidence that the lack of sodium chloride so alters the cell surfaces that subsequent exposure to high concentrations of sodium chloride results in induction comes from a few experiments recorded in Table IX. Experiment 1 shows the effect of substitution of glycine for sodium chloride. Short exposures to this solution result in the induction of spreading nerve after the aggregates are returned to high concentrations of sodium chloride. When ethylene glycol is substituted for sodium chloride the cells do not survive, but when ethylene glycol is combined with a low concentration of sodium chloride induction of nerve occurs when the cells are returned to a high concentration of sodium chloride (exp. 2). When ethylene glycol is added to a high concentration of sodium chloride there is no inductive activity of the solution. Thus, again the alteration of cell surfaces

appears to be the result of low sodium chloride content rather than the action of ethylene glycol itself.

Finally in experiment 3 the cells are exposed to low sodium chloride content and lack of sodium chloride for short intervals and then cultured in high concentrations of sodium chloride. After both treatments nerve is induced, indicating that the very low concentrations of sodium chloride result in some alteration in the cell surfaces so that induction occurs after the cells are returned to the higher concentration of sodium chloride. It is interesting that the treatment with lack of sodium chloride results in the same type of induction as with sucrose (experiment 3).

Discussion

Clearly substances such as sucrose do not induce by themselves but rather prepare the cells for induction by the salt solution in which they are cultured. The induction by the salt solution is proportional to the concentration of sodium chloride. Do all or most of the so-called inductors act in the same manner as sucrose? Since all the experiments on induction have been carried out in Holtfreter's solution containing a concentration of sodium chloride of 3.4 mg./ml., possibly all the inductions obtained by various compounds and mixtures may be attributed to Holtfreter's solution.

The above possibility is reinforced by the investigations of Barth (1941) and of Holtfreter (1944), which show that Holtfreter's solution will induce neural tissue in *Ambystoma maculatum* and *Ambystoma opacum* presumptive epidermis without benefit of additives of any sort. This is a clear-cut demonstration that Holtfreter's solution is an adequate inductor of nervous tissue. Thus, any compound or mixture claimed as an inductor when used in Holtfreter's solution or its equivalent may merely be preparing the cells for induction by the salt solutions. In order to prove that any substance is an inductor it will be necessary to show that induction occurs during the time of treatment and not after the cells are returned to a salt solution.

In view of the wide variety of compounds and mixtures which have been claimed as inductors, it does seem more reasonable to suppose that all induction is brought about by the ions in the salt solutions used for the culture of presumptive epidermis. If so, we can begin to think more clearly about the mechanism of induction by ions instead of trying to make sense out of the disorderly array of so-called inductors.

As far as normal induction by the mesoderm is concerned, it may now be suggested that the mechanism of normal induction is by ways of ions. For example, we find that cultures of mesoderm mixed with ectoderm prepared from lateral blastoporal lips contain functional nerve and pigment cells as well as muscle and mesenchyme. Thus, mesoderm induces nerve and pigment cells under the conditions of our experiments. However, if the sodium chloride content of our salt solution is reduced to 2.57 mg./ml. no nerve nor pigment cells are induced but muscle and mesenchyme differentiate normally. Thus, the normal induction of nerve and pigment cells by mesoderm is dependent upon a high concentration of sodium chloride. This suggests that the role of the mesoderm during normal gastrulation is to prepare the presumptive neural plate for induction by the ions

present in the blastocoel fluid. This preparation may simply consist in an increase in permeability so that the ions penetrate. Experiments designed to test the above suggestion are in progress.

If we accept the idea that basically induction is brought about by ions, then we have first the problem of which ions in the salt solution are necessary. Previous experiments have shown that Na^+, K^+, Ca^{++}, Mg^{++} and HCO_3^- can induce (Barth and Barth, 1963, 1964 and 1965). Secondly, how do the ions act inside the cell to induce cellular differentiation? A previous study of ion induction (Barth, 1965) showed a correlation between the effects of ions as inductors and their effects on the electrophoretic mobility of DNA. Possibly the ions in our salt solution act directly upon DNA complexes.

Summary

1. An analysis of the mode of action of sucrose as an inductor of the presumptive epidermis of the *Rana pipiens* gastrula leads to the conclusion that sodium chloride is the actual inductor.

2. After treatment with sucrose, induction is proportional to the concentration of sodium chloride in the culture medium. After treatment with sucrose, induction is proportional to the length of exposure to a solution containing 3.4 to 5.15 mg. sodium chloride per ml.

3. It is concluded that sodium chloride in concentrations of from 3.4 to 5.15 mg./ml. is an adequate inductor, while in concentrations from 2.00 to 2.57 mg./ml. sodium chloride does not induce but will sustain the differentiation of various cell types after induction.

4. It is suggested that normal induction by the mesoderm during gastrulation may be brought about by the ions present in the blastocoel. The hypothesis that ions act directly upon DNA complexes has been advanced in a previous paper on induction.

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